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Fumigant Toxicity is the Major Route of Insecticidal Activity of Citruspeel Essential Oils

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Abstract: Dosages (>10 ml mg^{-1} against *Callosobruchus maculatus* F. or *Sitophilus zeamais* Motsch; >20 ml kg^{-1} against *Dermestes maculatus* Deg.) of citruspeel oils reduced oviposition or larval emergence through parental adult mortality, but had no residual activity on the eggs or larvae produced by survivors. Oil-treated grains (7 ml kg^{-1} against *C. maculatus*) or dried fish (28 ml kg^{-1} against *D. maculatus*) which caused 100% mortality 1 h after application lost all activity within 24 h, thus confirming the non-residual nature of the effects. The activity of limepeel oil against test insects was found to be dependent on the time interval between the application of oil and start of bioassays. The non-volatile residues of limepeel oil were not toxic to insects on glass and dried-fish surfaces.

Topical toxicity trials against *D. maculatus* adults also illustrated the relative unimportance of contact toxicity of citrus oils, as appreciable mortality (at application rates of up to 2 μl per insect) was obtained only when treated insects were confined in air-tight glass chambers. The volatility of toxic constituents in the oils was further illustrated by mortality of untreated *C. maculatus* adults confined in air-tight chambers with topically treated *D. maculatus*. A more efficient way to use citruspeel essential oils to control insects would be as a fumigant in relatively enclosed or air-tight systems.

Key words: fumigant, insecticidal citruspeel, oils.

1 INTRODUCTION

There is an increasing interest in plant and microbial products as sources of insecticides, due to the environmental and toxicological side effects of many synthetic organic insecticides,[1–3] and to the often widespread resistance to them.[4–6] Many developing countries cannot afford to import the newer, more expensive and sometimes less environmentally damaging pesticides. It is worthwhile therefore to look for alternative sources of pesticides in the regions concerned. Extracts from edible plant products that show insecticidal activity could therefore be important since they may be less toxic to man and livestock. The modes of activity of any insecticidally active extracts are worthy of investigation to establish the most efficient ways to using them to achieve pest control.

In contrast to studies with fixed vegetable oils,[7,8] less interest has been shown in citrus essential oils, but recently, attempts have been made to use them to control storage insect pests.[9–12] Although one earlier worker[13] has observed a vapour toxicity of materials exuded from scarified citruspeels against insects, no work has established the greater importance of fumigant toxicity over residual contact activity of this group of essential oils. Most workers, including H.C.F. Su have always emphasised contact residual toxicity and never the fumigant toxicity of the oils against stored product pest.[12–16]

Recent literature reports have evaluated only the contact toxicity of citrus essential oils and their principal components against storage pests like *Callosobruchus rhodesianus*,[17] *C. maculatus* F.,[17] *Sitophilus oryzae* L.[18] and *Tribolium confusum* Du Val[19] by applying the oils to grains or topically onto adult insects.

The work reported in this paper was aimed at demonstrating the relative importance of fumigant toxicity of citruspeel oils compared with their residual

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contact toxicity, in order to establish the most effective mode of employing these locally available oils in pest-control systems.

2 EXPERIMENTAL METHODS

2.1 Test compounds

Industrially extracted citruspeel oils were obtained from Zimmermann Hobbs Ltd, UK.

Industrially extracted groundnut oil was obtained from a Lagos supermarket and served as reference fixed vegetable oil (without essences).

2.2 Insect cultures

Stock cultures of strains of *Callosobruchus maculatus* F., *Sitophilus zeamais* Motsch. and *Dermestes maculatus* Deg. were obtained from the Overseas Development Natural Resources Institute (ODNRI), UK, where they had been isolated from all kinds of insecticide for several years. Subsequent cultures of *C. maculatus* and *S. zeamais* held separately in jam-jars (880 ml) were reared on black-eyed beans (cowpea, 350 g per jar) and wheat (350 g per jar) respectively. Cultures of *D. maculatus* were established by adding about 50 young adult beetles to a mixture of fishmeal (400 g) and dried yeast (25 g) in a Kilner jar (2·7 litres). A wad of wet cotton wool was placed at the bottom of the jar to encourage oviposition.[20] After three weeks the parental adults were removed from the culture medium and the jars resealed with filter paper secured by paraffin wax.

All insect cultures were kept at 28°C and 70% RH under constant red light. All bioassays were conducted under these same conditions of temperature, humidity and light.

2.3 Rate of loss of volatile constituents of plant oils

In an initial experiment, weighed amounts of limepeel or groundnut oil (1 ml) were added to an aluminium foil container($5 \times 5 \times 0{\cdot}5$ cm^3) and placed in the room where bioassays were conducted (28°C) reweighing at hourly intervals for the first 6 h and then once every 24 h for 15 days.

In a second experiment, the rates of evaporation of limepeel oil and groundnut oil were compared by pipetting 0·1 ml of each oil separately on to a pre-weighed Whatman No. 1 filter paper, 11 cm diameter, which was suspended in the bioassay room and reweighed at 6 h and 24 h.

2.4 Tests on *Callosobruchus maculatus* on cowpeas

2.4.1 Contact activity of citruspeel oils

Citruspeel oils (lime, tangerine, grapefruit) were applied separately at concentrations of 3·5 and 14 ml kg^{-1} seed to cowpea grains by mixing in a Griffin flask shaker running for 15 min. This procedure for oil application to grains (cowpea or wheat) was adopted throughout the work, except where otherwise stated. Grains used as controls were similarly shaken but no oil was added. Six to eight hours after oil application, four one to four-day-old *C. maculatus* adults (two females, two males) were confined with 20 treated or untreated cowpea grains for five days. All treatments were replicated six times.

When parental mortality had been assessed, cowpeas were transfered into ventilated glass vials and the total number of eggs laid by *C. maculatus* assessed 12 days after the end of the oviposition period. This was achieved by inspecting each cowpea grain under a binocular microscope ($\times 8$) and counting *C. maculatus* eggs laid on the surfaces of each seed. Adult emergence was assessed daily for 19 days following the date on which the earliest emergence was observed, to prevent overlap of first and second generations.

To test further the acute toxicity of citruspeel oils, the experiments described above were repeated, but this time adult insects (15 or 20 insects per replicate) were exposed to several concentrations of oil (0·86 to 7·0 ml kg^{-1}) after application, and mortality assessed 24 h later.

2.4.2 Residual activity of citruspeel oils

The rate of loss of biological activity was tested by comparing the effects of oil on adult mortality and progeny development, soon after oil application and after a time lag (24 h). The non-volatile groundnut oil, known to have long residual ovicidal activity, was used as a reference compound.

Limepeel and groundnut oils were separately applied to cowpea grains at 7 ml kg^{-1} confining four one to three-day-old *C. maculatus* adults (two females, two males) with 20 treated or untreated grains in Petri dishes for five days. Each treatment was replicated three times. There were two sets of bioassays for each oil, initiated 1 h and 24 h after oil application. Bioactivity of the oil was assessed by adult mortality after 24 and 48 h, number of eggs laid on grains, and adult emergence

2.4.3 Toxicity of non-volatile citruspeel oil residues

In order to establish that biological activity resides mainly in the volatile components of citruspeel oils, the oils were exposed on a non-absorbent surface to allow evaporation of volatile components before exposing insects to the non-volatile residues to measure mortality. Limepeel and orangepeel oils (0·1 ml) were separately brushed over the surfaces of a glass Petri dish (7 cm dia.) to give a thin film of oil; leaving the dishes open for 72 h at 30 32°C allowed volatile constituents to evaporate. Fifteen zero-to one-day-old *C. maculatus* adults (mixed sexes) were then confined on the oil-

treated glass surfaces and mortality assessed after 24, 48 and 120 h. All treatments were replicated six times.

2.5 Test on *Sitophila zeamais* on wheat

2.5.1 Contact activity of citruspeel oils

Citruspeel oils were applied separately to wheat as described for *C. maculatus* on cowpea in Section 2.4.1. six to eight hours after oil application, six three to nine-day-old *S. zeamais* adults (three female, three male) were confined with 10 g of treated or untreated wheat grains for 16 days in glass Petri dishes (7 cm dia.). All treatments were replicated six times. Since *S. zeamais* lays its eggs inside the wheat grain, oviposition was not assessed. Parental adult mortality was assessed after the oviposition period, the grains were transferred into ventilated vials, and adult emergence was assessed daily for 23 days from when the earliest emergence was observed.

To test further the acute contact toxicity of citruspeel oils against another grain-eating beetle, the experiment described above was repeated but this time adult insects (15 or 20 per replicate) were exposed to several concentrations (1·75–10·5 ml kg^{-1}); 1–2 h after oil application, and mortality assessed 24 h later

2.6 Tests on *Dermestes maculatus* on dried fish

2.6.1 Contact activity of citruspeel oils

Citruspeel oils were applied to dried fish (*Salmo gairdneri* Rich. prepared and dried after procedures published earlier[20]) by brushing the required amounts of oil to give concentrations of 28 and 56 ml kg^{-1} onto skin and muscle surfaces of the fish strips; using a size 3 camel-hair brush. About 8 h after oil application; four one-to 12-day old *D. maculatus* adults (two females, two males) were confined in ventilated honey jars with treated or untreated dried fish strips (15 g) for 10 days. All treatments were replicated six times. After the oviposition period, parental adults still alive were assessed and removed; also the number of emergent larvae (18 days after start of bioassays) and subsequently, adults (removed daily to mimimise post-emergent feeding) were recorded. In addition all emergent larvae, together with the fish strips, were weighed separately.

To test the acute contact toxicity of the citruspeel oils against adults, the experiment described above was repeated, but, this time, adult insects (15 or 20 per replicate) were exposed to several concentrations of oils (7–5 ml kg^{-1}) 1–2 h after oil application and mortality assessed 24 h later.

2.6.2 Loss of activity in contact toxicity of citruspeel oils

If evaporative loss of volatile components in citruspeel oils reduces contact toxicity, the topical application of equal volumes of oil should cause higher mortality in treated insects that are confined in air-tight chambers than in open chambers. To test this hypothesis, limepeel oil was topically applied to the dorsal prothoracic region of one-to 15-day-old adult *D. maculatus* (mixed sexes; large enough for topical application) using an Arnold Micro-applicator fitted with a glass Aglar Syringe (1 ml). Thirty insects were dosed per treatment at 0·5, 1·0 and 2·0 μl per insect, handling the control insects similarly but without dosing. Two sets of insects were treated as described above; one set was left in open honey jars (15 insects per jar) while the other set of similar treatments was placed in air-tight glass chambers (500 ml, also 15 insects per chamber) immediately after topical application. Mortality was assessed after 24 h.

Secondly, to test whether the components of citruspeel oil which evaporated from the topically dosed insects were biologically active, 15 untreated *C. maculatus* adults (for ease of differentiation) were confined in air-tight glass chambers with five *D. maculatus* adults which had been topically treated with limepeel oil (1 μl per insect), and the mortality of each species was assessed after 24 h.

In order to ascertain that it was the volatile components of oil lost via evaporation that caused mortality in *C. maculatus* confined with topically treated *D. maculatus*, 15 untreated *C. maculatus* adults were fumigated with limepeel oil at 5 μl per 500 ml glass chamber (oil applied onto filter paper which was hung inside an air-tight glass chamber). All treatments, including controls (untreated *C. maculatus* and *D. maculatus* adults confined in the same air-tight glass chamber and in separate chambers) were replicated twice and mortality was assessed after 24 h.

2.6.3 Vapour toxicity of citruspeel oils

To substantiate further the vapour toxicity of the volatile components of citruspeel oil, adult *D. maculatus* insects were fumigated in air-tight glass chambers (500 ml) with 5 μl of limepeel oil applied to filter paper (3 cm dia.) left hanging inside the chamber. To test further the lower efficiency of contact toxicity with the test oils, another set of five adult *D. maculatus* were treated topically with the oil at 1 μl per insect (total of 5 μl on cuticles of five insects per chamber) and confined in similar glass chambers. Each treatment was replicated six times (five insects per replicate), assessing mortality 24 h later.

2.6.4 Residual activity of citruspeel oils

The residual activity of citruspeel oil against *D. maculatus* was measured by evaluating the rate of loss of biological activity by carrying out an experiment similar to the one described in Section 2.4.2 Limepeel and groundnut oil (non-volatile reference oil) were separately applied to dried fish strips at 28 ml kg^{-1}, confining 20

zero-to 15-day-old adults (mixed sexes) with treated or untreated fish strips (10 g) for 10 days. Each treatment was replicated three times. Three sets of bioassays for each oil were set up and initiated 1 h, 24 h and 240 h after oil application. Bioactivity of the oils for each post-application period was assessed by adult mortality after 24 h and 48 h from the start of the bioassay, number of emerged larvae (18 days after start of bioassay), and adult emergence.

2.6.5 Toxicity of non-volatile citruspeel oil residues
Batches of fish strips (10 g) were immersed in limepeel oil for 30 s and then placed in a ventilated oven at 34°C for 96 h in order to remove volatile fractions of the oil. Untreated control fish strips were similarly treated in a separate oven and along with treated strips were allowed to re-equilibrate in plastic dishes kept in the bioassay room for seven days. In the bioassay, four one-to 15-day-old *D. maculatus* adults (two females, two males), were confined with treated or untreated fish strips for seven days. All treatments were replicated three times. The experiment was evaluated by assessing the number of larvae (18 days after the start) and emergent adults.

2.7 Statistics

All dose–response (mortality) data were analysed using a computer package for probit analysis which included tests for parallelism and relative potency based on accepted procedures.[21]

Data on progeny development were analysed by analysis of variance (ANOVA). Wherever ANOVA was used in this work, the first test consisted of comparing all treatment means including control. Further analysis was carried out only if there was a significant difference at the 5% level ($P < 0.05$ as a minimum requirement). Comparison of individual means was based on least significant differences (LSD) at $P < 0.05$, 0.01 and 0.001.

3 RESULTS

3.1 Rate of loss of volatile constituents of plant oils

Seventy percent by weight of limepeel oil was lost within 6 h of exposure at 28°C, and after the seventh day of exposure the weight of the citruspeel oil remained constant, having lost 90% by weight. All the other citruspeel oils (grapefruit, lemon, lime and tangerine) tested showed a similar rapid loss of volatile constituents by evaporation when exposed at 28°C (room temperature). In contrast, groundnut oil (fixed vegetable oil, no volatile constituents or essences) also exposed at 28°C, lost no measurable weight over the 15-day period.

3.2 Tests on *Callosobruchus maculatus* on cowpeas

3.2.1 Contact activity of citruspeel oils
Limepeel and tangerinepeel oils at 14 ml kg^{-1} significantly ($P < 0.05$) reduced oviposition of *C. maculatus* when compared with control (Table 1). The citrus oils significantly ($P < 0.05$) reduced adult emergence when compared with control only in treatments where there had been corresponding significant reduction in oviposition (Table 1). The overall percentage of *C. maculatus* adult emergency was not significantly different from control (employing a genstat ANOVA model with added controls and with substituted predicted values for all zero values).

When acute toxicity bioassays started 1 h after application to the substrate (cowpeas), the two citruspeel oils tested had similar levels of toxicity against *C. maculatus*,

TABLE 1
Effects of Citruspeel Oils on Oviposition and Adult Emergence of *Callosobruchus maculatus* and *Sitophila zeamais*[a]

		C. maculatus			
Treatment	(*ml* kg^{-1})	x̄ *No. of eggs laid*[b] *(oviposition)*	x̄ *No. of emerged adults*[b]	x̄ *adult emergence (%)*[b]	S. zeamais x̄ *adult emergence*[b]
Control		126·8	84·5	67·0	69·5
Limepeel oil	(3·5)	85·3	59·2	69·0	68·5
	(14)	0·0***	0·0***	0·0	15·2***
Tangerinepeel oil	(3·5)	116·7	82·7	71·0	44·0**
	(14)	72·0*	45·0**	63·0	39·2***
Grapefruitpeel oil	(3·5)	149·0	82·0	55·0	62·8
	(14)	88·5	38·7**	44·0	60·0

[a] $n = 6$.
[b] *, **, *** Significantly different from control at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

TABLE 2
Relative Toxicity of Citrus Oils on Grains or Dried Fish against *Callosobruchus maculatus*, *Sitophila zeamais* and *Dermestes maculatus* Adults

	24-h Toxicity (ml kg^{-1})				
Treatments	LC_{50} *(95% CL)*	LC_{95} *(95% CL)*	*Slope (±SE)*	χ^2	*DF*
C. maculatus on cowpea					
Tangerinepeel oil	4·49 (2·84–11·95)	9·85 (7·49–15·67)	4·16 (±0·52)[a]	6·15	2
Limepeel oil	2·83 (2·60–3·07)	5·73 (5·04–6·85)	5·37 (±0·52)[a]	3·68	2
S. zeamais on wheat					
Limepeel oil	4·15 (3·90–4·40)	6·39 (5·88–7·16)	8·75 (±0·88)[a]	7·60	3
Tangerinepeel oil	5·04 (4·65–5·45)	9·14 (8·13–10·77)	6·37 (±0·66)[a]	0·77	1
D. maculatus on dried fish					
Limepeel oil	14·31 (12·55–16·0)	38·88 (33·16–48·36)	3·79 (±0·37)	1·65	1

[a] Pairs of data do not contradict the hypothesis of parallelism.

with overlaps in 95% confidence limits of LC_{50} values (Table 2).

3.2.2 Residual activity of citruspeel oils
In bioassays which started 1 h after application of limepeel oil to cowpea grains, there was 100% adult mortality for *C. maculatus* and no progeny development was observed. In contrast, when the bioassay began 24 h after citruspeel oil application, there was no appreciable adult mortality and progeny development was comparable with that of control (Table 3). When groundnut oil was applied to cowpeas against *C. maculatus*, there was no differential effect on adult mortality or progeny development when bioassays began 1 h and 24 h after application (Table 3).

3.2.3 Toxicity of non-volatile citruspeel oil residues
The non-volatile residues of limepeel and orangepeel oils on glass surfaces were not found to be active against *C. maculatus* adults. After 120 h of exposure to glass surfaces treated with residues of limepeel and orangepeel oils only 6% and 13%, respectively, of insects ($n = 90$) were found dead and this was similar to the mortality (13%) of insects on untreated surfaces (control).

3.3 Tests on *Sitophila zeamais* on wheat

3.3.1 Contact activity of citruspeel oils
Only limepeel oil at 14 ml kg^{-1} and tangerinepeel oil at 3·5 and 14 ml kg^{-1} significantly reduced *S. zeamais* adult emergence compared with controls (Table 1).

When applied to wheat grains, the two citruspeel oils tested had similar levels of toxicity against *S. zeamais* adults (with overlaps in 95% C.L. of LC_{50} values; Table 2).

3.4 Tests on *Dermestes maculatus* on dried fish

3.4.1 Contact activity of citruspeel oils
Statistical comparison of the treatment means showed that only oil applications at 56 ml kg^{-1} significantly ($P < 0·001$) reduced emergent *D. maculatus* larvae compared with control (Table 4). The results on larval survival into adults show a trend towards a higher

TABLE 3
Effect of the Time Interval between Oil Application and Introduction of Insects on the Toxicity of Oils against *Callosobruchus maculatus*[a]

Treatment	*Time interval (h)*	*Mortality of parental adult (out of 20) (at 24 h)*	$\bar{x}$ *eggs laid*	$\bar{x}$ *adult emergence*	$\bar{x}$ *adult emergence %*
Control		0	128·6	89·6	69·7
Groundnut oil[b]	(1)	0	45·6	36·0	79·0
	(24)	0	38·0	35·0	92·0
Limepeel oil[b]	(1)	20	0·0	0·0	0·0
	(24)	1	84·0	46·0	55·0

[a] $n = 5$ (4 insects per replicate).
[b] Oils applied at 7 ml kg^{-1}.

TABLE 4
Effects of Citruspeel Oils on Larval and Adult Emergence of *Dermestes maculatus*[a]

Treatments	(*ml kg*$^{-1}$)	$\bar{x}$ *No. of emerged larvae*[b]	$\bar{x}$ *No. of emerged adults*[b]	$\bar{x}$ *adult emergence* (%)
Control		69·0	49·0	71
Limepeel oil	(28)	58·8	44·8	76
	(56)	5·2***	4·3***	83
Orangepeel oil	(28)	68·2	40·7	60
	(56)	28·2***	22·3***	79
Grapefruitpeel oil	(28)	66·5	41·0	62
	(56)	25·7***	19·8***	77

[a] $n = 6$.
[b] **, *** Significantly different from control at $P < 0{\cdot}01$ and $P < 0{\cdot}001$ respectively.

percentage adult emergence at higher dosages, although the overall effects of the oils were not significantly ($P > 0{\cdot}05$) different from controls (Table 4). Additionally, the citruspeel oils were found to have no significant ($P > 0{\cdot}05$) effect on weight of fish consumed per larva (Table 5).

Limepeel oil, the only oil tested against *D. maculatus* adults (Table 2), was less active against this species at the LC_{50} level (no overlaps in 95% C.L.) than it was against *C. maculatus* or *S. zeamais adults*

3.4.2 Loss of activity in contact toxicity of citruspeel oils

Topically applied limepeel oil was shown to be more active against adult *D. maculatus* when treated insects were enclosed in an air-tight chamber (24 h LC_{50} = 0·65 µl per insect) than when the insects were left in an open jar (24 h $LC_{50} > 2{\cdot}0$ µl per insect; size of insect did not permit application of dosages above 2 µl per insect). It was also found that, when this citruspeel oil was topically applied to adult *D. maculatus*, sufficient oil vaporised to give 100% mortality of *C. maculatus* confined in the same fumigation chamber. There was also 100% mortality of *C. maculatus* adults when confined alone inside chambers containing citrus oil vapour that evaporated from the surface of filter paper.

3.4.3 Vapour toxicity of citruspeel oils

The vapour toxicity of limepeel oil (5 µl) applied to filter paper against *D. maculatus* within an air-tight glass chamber (500 ml) was significantly ($P < 0{\cdot}001$) more than that of the same total amount of oil topically applied (at 1 µl per insect to five *D. maculatus* adults) within the same size glass chamber (Table 6).

3.4.4 Residual activity of citruspeel oils

In bioassays that began 1 h after application of limepeel oil to fish strips there was over 98% adult mortality and only three emergent adults were observed. In contrast, when the bioassays began 24 h or 240 h after application of the citruspeel oil, there was significantly lower or no adult mortality accompanied by significantly higher progeny development, comparable with that of the untreated control (Table 7).

2.4.5 Activity of non-volatile citruspeel oil residues

Non-volatile residues of limepeel oil on dried fish strips had no significant ($P > 0{\cdot}05$) effect on progeny development of *D. maculatus* based on mean number of emer-

TABLE 5
Effects of Citruspeel Oils on *Dermestes maculatus* Larval Weight and Larval Consumption of Fish during the First 18 Days of Bioassay[a]

Treatment oils	(*ml kg*$^{-1}$)	$\bar{x}$ *weight of larva*[b] (*mg*)	$\bar{x}$ *amount of fish consumed per larva* (*mg*)
Control		12·7	39·0
Limepeel	(28)	10·5	33·8
	(56)	5·0*	23·0
Tangerinepeel	(28)	8·3	30·0
	(56)	8·5	32·0
Grapefruitpeel	(28)	8·5	29·0
	(56)	6·0*	30·0

[a] $n = 6$
[b] Significantly different from control at $P < 0{\cdot}05$.

TABLE 6
Comparative Toxicity of Limepeel Oil when Applied Topically or as a Vapour to *Dermestes maculatus* adults[a]

Treatment	*24-h mortality*[b]
Topical[c]	3***
Vapour[d]	16
Control	0

[a] $n = 30$
[b] *** Significantly different from vapour treatment at $P < 0{\cdot}001$ (2×2 contingency Chi-square test).
[c] 1 μl per insect.
[d] 5 μl per chamber applied on filter paper.

gent larvae: 30·3 (control), 28·0 (limepeel oil) and adult emergence: 48·3% (control) and 50% (limepeel oil).

4 DISCUSSION

Experiments on *C. maculatus*, *S. zeamais* and *D. maculatus* showed that, wherever citruspeel oils caused adult mortality, few or no eggs and emergent larvae/adults, or none at all, were produced. In treatments which caused no adult mortality, oviposition or larval emergence appeared to be normal. This suggested that the citrus oils reduced oviposition by causing rapid adult mortality.

It was shown that the percentage adult emergence in citrus oil treatments was not significantly different from that of the control, illustrated particularly by the data for *C. maculatus*. Thus citrus oils appear to act against progeny development by reducing oviposition through adult mortality, but have little or no residual activity on eggs or larvae. This suggestion appears to be in conflict with reports of earlier workers,[15] which showed that the insecticidal activity of the citrus oils they tested lasted for over 300 days on grains.

However, these authors applied the non-volatile fraction of citrus oils at 1% by weight of cowpeas. In that case, the oil residue may have remained as a film on the seed coat and continued to act against eggs by reducing their gaseous exchange in a similar way to fixed vegetable oils such as groundnut oil.[8] Although contact toxicity was demonstrated against the three test insect species in the present work (as in earlier work[14,15]), it was also found that oil-treated grains or dried fish which caused 100% mortality 1 h after application lost all activity within 24 h. These results together confirm the non-residual nature of the insecticidal properties of citrus oils, in contrast to the persistent activity demonstrated for groundnut oil, a fixed vegetable oil. The weak contact toxicity of citrus oils is further illustrated by the fact that toxicity levels (LC_{50} values) against adult insects were in ml litre^{-1}, whereas measurement as low as 10 μl litre^{-1} were shown in this series of experiments to cause 100% mortality to *D. maculatus* adults in minature glass fumigation chambers.

Topical toxicity trials also illustrated the relative unimportance of the contact toxicity of citrus oils, as appreciable mortality at application rates of up to 2 μl per insect was obtained only when treated insects (*D. maculatus*) were confined in air-tight glass chambers. Thus, the toxic volatile components of the oil apparently evaporated into the surrounding air space faster than they penetrated the insect cuticle on contact. This assumption is further substantiated by the mortality of untreated *C. maculatus* adults confined in air-tight chambers with topically treated *D. maculatus* adults. Therefore, earlier workers,[13,14,22] who measured the topical toxicity of citrus and non-citrus essential oils in open systems, may have measured only a fraction of their true toxicity. Contact toxicity for these types of plant oil is thus rather incidental and only effective before the toxic volatiles evaporate fully from the surfaces of application. Certainly, no evidence was obtained in this work for toxic non-volatile limepeel oil residues on either glass or dried fish.

All the citruspeel oils tested in this study lost over 90% by weight through evaporation from filter paper in 6 h at 28°C and 70% RH in the open bioassay room, unlike fixed vegetable oils, typified by groundnut oil, which lost no measurable weight over a period of 15 days of observation. This volatility explains why the

TABLE 7
Effect of the Time Interval between Oil Application and Introduction of Insects on the Toxicity of Limepeel Oil against *Dermestes maculatus*[a]

Treatment	*Time interval (h)*	*Mortality of adults (out of 60) at 48 h*	*x̄ emergent larvae*
Control		0	145
Limepeel oil[b]	(1)	59	3
	(24)	23	108
	(240)	0	155

[a] $n = 3$ (20 insects [mixed sexes] per replicate).
[b] Oil applied at 28 ml kg^{-1}.

activity of limepeel oil against *C. maculatus* and *D. maculatus* was found to be dependent on the time interval between the application of the oil and the start of the bioassay.

The experiments reported above show that a more effective way to use citruspeel essential oils to control insects would be as a fumigant in a relatively enclosed or air-tight system. In direct contrast, fixed vegetable oils such as groundnut oil (which do not have volatile components) showed an activity against eggs of test insects which was independent of the time interval between application and start of the bioassay and this type of oil can only be used as a contact ovicide.[8,22]

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